# LIPIDS OF PISTIL EXUDATES AND SELF INCOMPATIBILITY IN *LILIUM LONGIFLORUM* THUNB.

by

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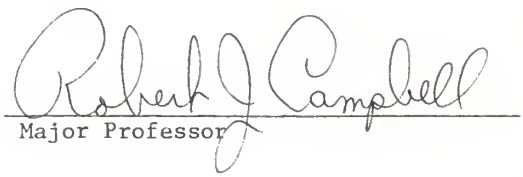

Major Professor

TABLE OF CONTENTS

List of Figures

# List of Tables

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## INTRODUCTORY STATEMENT

This thesis has been written in manuscript form, for submission for publication in Theoretical and Applied Genetics. This research was conducted from the spring of 1976 through the fall of 1977, in the Department of Horticulture laboratories at Kansas State University.

# Fatty Acids and Lipid Classes in Stigmatic Exudate and Materials Flushed from the Stylar Canals of Heat-treated, DMSO-treated and Aged Styles of *Lilium longiflorum* Thunb. 'Ace' and 'Nellie White'[1]

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Summary. Thin-layer chromatography of lipid classes of stigmatic exudate using a solvent system composed of chloroform: methanol:water (65:25:4) resulted in 2 spots with $R_f$ values of 0.93 and 1.0, while the 3 stylar flushes each had spots with $R_f$ values of 0.75, 0.93 and 1.0. Thin-layer chromatography of lipid classes from stigmatic exudate and the 3 stylar flushes using hexane:diethyl-either:acetic acid (90:10:1) resulted in 11 spots for each extract; however, some spots were different. Gas chromatographic analysis of fatty acids from *L. longiflorum* stigmatic exudate revealed 23 peaks, while materials flushed from the hollow style of heat-treated, DMSO-treated and aged styles produced 21 peaks.

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INTRODUCTION

Gametophytic self incompatibility enforces outbreeding in Angiosperms. The genetic mechanism (de Nettancourt 1972), controlled by the 'S' locus allows a pollen grain to germinate and accomplish fertilization if the 'S' allele it carries is not present in the diploid tissue of the female organs (stigma and style). The Easter lily, Lilium longiflorum, is self incompatible. Incompatible pollen tubes germinate and penetrate into the style but grow at such a slow rate so as to fail to reach the ovary before floral senescence. After 48 hr growth in styles at room temperature, incompatible pollen tubes average 45 mm in length, while compatible pollen tubes average 85 mm (Ascher and Peloquin 1970).

The hollow pistil of L. longiflorum Thunb. produces two kinds of exudates, stigmatic and stylar. Stigmatic exudates are produced by many flowering and important horticultural crops, but vary in composition and quantity secreted (Martin 1969). The many chemical compounds in stigmatic exudates may be involved in a variety of functions such as protecting the stigma from dessication, rain, insects, fungi and could possibly inhibit foreigh pollen from germinating (Linskens 1966). However, the main purpose of the stigmatic exudate seems to be that of providing an environment suitable for pollen germination. The stigma of L. longiflorum from the day after anthesis until floral senescence produces large amounts of stigmatic exudate. This stigmatic exudate is sticky evidently helping pollen adhere. It is also seemingly a complex mixture of compounds capable of serving as a better growth medium for in vitro pollen tube growth in lily and other species than most complex artificial medium (Campbell 1975).

Organic solvents have been used frequently to remove stigmatic secretions from the stigma. This technique may remove substances other than

stigmatic secretions and requires a large number of flowers to collect enough exudate for biochemical analysis. In contrast, stigmatic exudate from the Easter lily can be collected using a hypodermic needle, resulting in a true stigmatic exudate, one which has not been subjected to organic solvents and can be precisely determined biochemically.

Stigmatic exudates are composed of complex mixtures of lipid and phenolic compounds (Martin 1969a, b, 1970b, c). However, amino acids (Konar and Linskens 1966) and sugars (Horovitz 1972) have also been found. The stigmatic exudate of L. longiflorum is a viscous, opaque, oily, whitish fluid, which contains free amino acids (Ascher unpublished data), carbohydrates and protein (Labarca et al., 1970). L. longiflorum styles labeled through the stem with 5-$^{3}$H-uridine contained label in the stigmatic exudate, however the molecular form is not a nucleic acid species (Campbell and Ascher 1975). Labeled stigmatic exudate collected from detached L. longiflorum pistils labeled with D-gulcose-1-$^{14}$C was fractionated on Sephadex G-100 and the polysaccharide component G-100-1 was injected into the hollow styles of nonlabeled detached pistils. Injected pistils were immediately cross pollinated with L. longiflorum pollen. Eighty four hr later, excised pollen tubes revealed that at least 25% of the carbohydrate substance in the pollen tubes was derived from G-100-1 (Loewus and Labarca 1972).

A longitudinal cut of the unpollinated, aged (1 day post-anthesis through floral senescence) style reveals a sticky, tightly appressed stylar exudate. This stylar secretion may provide nutrition for the growing pollen tube (Loweus and Labarca 1971). Pollen reserves aid in feeding the pollen tube temporarily but eventually the pollen tube needs an additional source of food to reach the ovary (Linskens 1964).

The nutritional role of stylar secretions in relation to pollen tube growth has been demonstrated biochemically. In lily styles labeled with myo-inositol-U-$^{14}C$, label was incorporated into pollen tube walls (Kroh et al., 1970). In another study, styles have been labeled with nucleic acid synthesis precursors, and the label secreted by the stylar tissue was found in pollen tube nucleic acids (Campbell and Ascher 1974).

Blowing air through the stylar canals of heat-treated, DMSO- treated and aged styles with an empty syringe and hypodermic needle produces bubbles at the ovarian end of the style. These bubbles may be the stylar exudate. The 3 stylar treatments each provide a check for the other, resulting in a more accurate biochemical analysis of stylar exudates.

Heat treatment of styles (aged 1 to 4 days post-anthesis) in a water bath at 50 C for 5 min (Table 1) removes the self-incompatibility reaction (Hopper, Ascher and Peloquin 1967). Pre-pollination heat treatment of styles followed by a stylar flush with 10 drops of redistilled water results in a loss of stylar capacity to support compatible pollen tube growth (Ascher 1975). The bubbling materials flushed from heat-treated styles is referred to as special stylar substance since on injection into heat-treated flushed styles, compatible growth is restored (Ascher 1975). The capacity to support compatible pollen tube growth is not restored in heat-treated, flushed styles by pre-pollination injection of stigmatic exudate (Ascher 1975).

Submerging styles in 5% DMSO for 10 min (aged 1 to 4 days post-anthesis) retards the growth of pollen tubes by 5 mm on incompatible pollination, but does not affect compatible pollen tubes (Ascher and Drewlow 1971). Dimethyl sulfoxide increases cell membrane permeability, and enters plant tissues rapidly (Garren 1967; Leonard 1967). Dimethyl

sulfoxide has also been used as a preservative for protein extraction and stabilization (Delmer and Mills 1969).

Compatible and incompatible pollen tube growth in detached styles of L. longiflorum varies with the physiological age of the style. In styles aged 5 to 6 days post-anthesis incompatible pollen tubes changes to compatible lengths (Ascher and Peloquin 1966). Secretion of stylar exudate is enhanced by aging.

Only materials flushed from heat-treated styles with air have been tested for the ability to restore compatible growth when injected into heat-treated flushed styles; materials flushed from DMSO-treated and aged styles of L. longiflorum have not been so tested; therefore, they cannot be rightly termed as special stylar substances. If each of the materials flushed from heat-treated, DMSO-treated and aged styles are special stylar substances, then the following assumptions can be made. Compounds common to the 3 stylar flushes might be the food necessary for compatible pollen tube growth, while compounds not in common may not be required for compatible growth. Differences in materials released into the stylar canal by the 3 stylar treatments should be due to differences in treatment. Specific biochemical patterns of the stigmatic exudate should differ from those of the 3 stylar flushes.

Gas liquid chromatograms of alditol acetates of neutral sugar hydrolyzates of stigmatic and canal exudates of L. longiflorum indicates differences in biochemical patterns. Stigmatic exudate contained rhamnose, arabinose, xylose, mannose, galactose and glucose while stylar flushes consisted of rhamnose, arabinose, galactose and glucose (Labarca and Loewus 1973). The stylar canal and stigmatic tissue of L. longiflorum are composed of morphologically different secretory cells (Yamada 1966, Rosen and Thomas 1970, Dashek et al, 1972), indicating potential differences in secretion products.

The purpose of this research was to determine the lipid classes and fatty acid composition of stigmatic exudate and materials flushed from the stylar canals of heat-treated, DMSO-treated and aged styles of L. longiflorum cultivars Ace and Nellie White.

MATERIALS AND METHODS

Flowers' from greenhouse grown plants of Lilium longiflorum Thunb. 'Ace' and 'Nellie White', cut and placed in water at room temperature before noon on the day of anthesis, provided the pistil exudates for this research. Stigmatic exudate was collected twice daily from flowers aged 1 day through floral senescence using a 25 gauge needle and syringe, stored in vials and frozen (-20C).

For collection of stylar exudates, styles were harvested from the flower by cutting through the ovary and placed on moistened filter paper in a petri plate. As an aid in the removal of the stylar exudates, a mixture of styles aged 1 to 4 days post-anthesis were either heat-treated by submerging in a 50 C water bath for 5 min or soaked in 10% DMSO for 10 min and the wet styles dried on paper toweling. Old styles were aged 5 to 6 days post-anthesis and utilized as a source of stylar exudate. Styles were then cooled to 10 C prior to flushing the canal.

Stylar exudates were removed from the canal by breaking off the remainder of the ovary and injecting 5% DMSO through the stigma using a 25 gauge needle and syringe until 5 drops (.16 ml) had fallen from the ovarian end of the style. The flushed materials were collected from 75 styles into a glass vial plunged into ice. Vials were stoppered with cork and frozen (-20C) for as long as a year until used.

Stigmatic exudate and materials flushed from the stylar canals of heat-treated, DMSO-treated and aged styles were extracted for total lipids with chloroform (analytical reagent):absolute methanol (2:1) in preparation for thin-layer and gas-liquid chromatographic analysis of lipid classes and fatty acids. Table 2 lists the different procedures used for extraction of total lipids. Extraction methods used for stigmatic exudate was altered for the stylar exudates due to the excess water

from the 5% DMSO. Also, thin-layer chromatography required more exudate than gas-liquid chromatographic analysis. The chloroform-methanol-exudate mixtures were homogenized in Virtis flasks at 45,000 rpm using a Virtis 45 homogenizer. The homogenate was filtered through a Buchner funnel into a 500 ml filtering glask, the residue on the filter paper (Whatman #1) washed with 10 ml of 2:1 chloroform-methanol, and the filtrate and wash combined. We then placed the emulsion into a separatory funnel, added distilled water so that the total volume of water was equal to ½ the amount of chloroform:methanol used, and shook the contents gently. Two layers formed in the separatory funnel after 5 to 15 min. The chloroform layer on the bottom of the separatory funnel is known to contain the lipids, while carbohydrates and proteins are drawn into the methanol-water layer on top. The chloroform layer was drawn into a 100 ml round-bottom flask and concentrated to near dryness (1 ml) under vacuum with a Buchler flash evaporator at 40 C. Preparation of the chloroform layer after extraction of stylar flushes for thin-layer chromatography required a removal of excess water prior to concentration. The chloroform layer was drawn into a 125 ml Erhlenmeyer flask containing a layer (20 gr) of anhydrous sodium sulfate that had been oven dried for 24 hr at 115 C. The chloroform extract was allowed to stand at room temperature for 2 hr.

For thin-layer chromatography the chloroform extract (1 ml) was applied as two concentrated spots (3 mm wide) 2.5 cm from the bottom edge of a 20 by 20 cm thin-layer plate of Silica Gel G (Mann), activated at 110 C for 10 min. Two standard mixtures were used, one containing monostearin; 1,2 and 1,3-distearin; and tristearin and the other a combination of oleic acid, triolein, cholesterol, methyl oleate and cholesterol oleate (Sigma). Lipid standards were spotted adjacent to and with the

lipid extract. Each component of the 2 lipid standards represents a particular lipid class. Within each class more than 1 compound can occur.

To determine the presence of polar lipids, chromatograms were developed to the top edge of the plate in a chromatographic chamber containing chloroform-methanol-water (65:25:4).

The presence of neutral lipids were determined using two thin-layer procedures. In one, neutral lipids in the chloroform extracts were separated using a solvent system composed of hexane:diethyl-ether:acetic acid (90:10:1), and the extract developed to the top edge of the plate (Stahl 1965). In another, the chromatographic plate was placed in a development chamber containing hexane:diethyl-ether (98:2), developed until the solvent front reached the top edge of the plate and then continued for an additional 10 min. After air drying, the plate was redeveloped in the same direction in a second tank containing hexane:diethyl-ether: acetic acid (50:50:1). When the solvent reached the upper edge, the plate was removed and air dried (Schlotzhauer et al., 1976).

The two-step one dimensional procedure for separation of plant neutral lipids was not used for separation of stylar extracts due to the inadequate separation of the lipid standards. A shortage of 'Ace' flowers prevented a cultivar comparison of materials flushed from the stylar canal.

Preparation of fatty acids for gas chromatographic analysis required an hydrolysis of the lipid extract into the component fatty acids and subsequent conversion to fatty acid methyl esters. The concentrated chloroform extract (1 ml), placed into a 20 ml test tube containing 10 ml of 2% $H_2SO_4$ in methanol, formed a miscible solution (reaction mixture), which was then heated in a shaker bath at 65-70 C for 1 hr. The test tube was allowed to cool until it could be held comfortably and the fatty acid methyl esters recovered by adding 1 ml hexane to the test tube and

mixing vigorously for 1 min with a glass stirring rod, resulting in 2 layers, the hexane layer on top and the reaction mixture on the bottom. Distilled water added to the test tube combined with the reaction mixture layer to bring the contents 2 cm from the top of the tube. Almost all of the hexane layer was transferred to a 10 ml test tube using a 0.5 ml pipette. The hexane layer was re-extracted twice by adding 1 ml volumes of hexane to the top of the test tube without disturbing the contents in the bottom layer and transferring each extract to the 10 ml test tube containing the initial hexane extract. The hexane extract was washed by pouring approximately 1 ml of distilled water into the test tube and, with the aid of a 22.8 cm disposable pipette, repeatedly sucking the water from the bottom of the test tube and spraying the hexane layer on top; the water was then removed from the test tube using the same pipette. The hexane extract was dried for 1 min by adding, with a spatula, approximately 2 gr anhydrous sodium sulfate to the 10 ml test tube. The hexane extract was then poured into a 3 dram vial, placed into a dessicator attached to a vacuum pump and concentrated to near dryness (0.5 ml) in preparation for gas-chromatographic analysis.

The hexane extract was introduced into the gas chromatograph (Varian Aerograph 600, stainless steel column, length 180 cm, filled with 10% diethylene glycol succinate on Chromosorb G, 80/100 mesh (Supelco), input temperature 200 C, column temperature 160-178 C, $N_2$ flow rate 25-40 ml/min., $H_2$ flow rate 25 ml/min., $O_2$ flow rate 300 ml/min., sample size 1-3 ul). Fatty acid methyl esters were identified using two standard mixtures, one containing oleic (18:0), palmitic (16:0), linoleic (18:2), linolenic (18:3) and stearic (18:0) acids, while the other included capric (10:0), caprylic (8:0), myristic (14:0), lauric (12:0) and palmitic acids (Sigma).

The retention times of fatty acids in each standard were plotted versus carbon number on log paper and used as a means of identification of unknown peaks. Saturated and unsaturated fatty acids were identified by taking the log of the retention time and plotting versus carbon number and the equivalent chain length values (ECL) compared to those of the 2 standards. Saturated odd carbon chain length fatty acids were identified by the ECL values in Table 3. Identification of unsaturated fatty acids utilized the ECL values as determined by Hofstetter, Sen and Holman (1965). Peaks with ECL values not matching up with known values were designated as unknown fatty acids. The entire procedure for lipid extraction and preparation for gas chromatographic analysis was carried through to completion without stigmatic exudate or the three stylar flushes, as a check for determining peaks due to contamination.

RESULTS

Thin-layer chromatographic analysis of total lipid extracts for polar lipids separated using a solvent system composed of chloroform: methanol:water (65:25:4) resulted in 2 spots with $R_f$ values of 0.93 and 1.0 for stigmatic exudate. The 3 stylar flushes had 3 spots with $R_f$ values of 0.75, 0.93 and 1.0. The spot at $R_f$ 0.75 was the result of DMSO, since the same spot appeared on thin-layer chromatographic plates spotted with extracts containing either a mixture of lipids from stigmatic exudate and DMSO or 5% DMSO.

Separation of lipid extracts from stigmatic exudate and of materials flushed from the stylar canal using a solvent system composed of hexane: diethyl-ether:acetic acid (90:10:1) provided a more suitable means for separating neutral lipids than did the two step, one dimensional procedure. Both stigmatic exudate (Fig. 1) and all stylar flushes (Fig. 2) resulted in 11 spots. The lipid patterns of stigmatic exudate did not vary between cultivars Ace and Nellie White. Differences were not found in the lipid patterns of heat-treated, DMSO-treated and aged styles. However stigmatic exudate produced 3 spots with $R_f$ values of 0.19, 0.23 and 0.49 which were not obtained for the 3 stylar flushes. The 4 different spots in the stylar flushes had $R_f$ values of 0.04, 0.06, 0.45 and 0.61. Lipid extracts from stigmatic exudate and materials flushed from the stylar canal revealed 6 spots each for which no $R_f$ values from lipid standards could be attributed. The $R_f$ values obtained from neutral lipids of stigmatic exudate and materials flushed from the stylar canal matched up closely with sterols, free fatty acids, triglycerides, methyl esters and sterol esters. Stylar flushes contained diglycerides as one not in common with stigmatic exudate.

The lipid extract of stigmatic exudate developed in one direction using a solvent system composed of chloroform:methanol (98:2) and subsequently in the same direction with chloroform:methanol:acetic acid (50:50:1) separated into 9 identical neutral lipid spots for each cultivar (Fig. 3). This two step, one dimensional procedure for separating plant neutral lipids did not separate all components of the 2 lipid standards adequately. The spots of methyl esters (methyl oleate) and the triglycerides (triolein and tristearin) apparently did not separate from each other as is the case when using Silica Gel H (Schlotzhauer et al., 1976). When the 2 standards were spotted separately, $R_f$ values for distearin (1,2 and 1,3) in one standard and cholesterol and oleic acid in the other could be determined but mixing the 2 standards resulted in only 2 spots. In spite of the undesirable separation, the neutral lipid spots of stigmatic exudate matched up with sterols, diglycerides, free fatty acids, triglycerides, methyl esters and sterol esters.

Gas chromatographic analysis of fatty acids resulted in 23 peaks for stigmatic exudate and 21 peaks for each of the materials flushed from the stylar canal of heat-treated, DMSO-treated and aged styles, ranging in carbon chain length from 8 to 18 (Table 3). Cultivar differences between 'Ace' and 'Nellie White' were not obtained for either sitgmatic exudate or stylar flushes. Materials flushed from the stylar canals of heat-treated, DMSO-treated and aged styles resulted in exactly the same fatty acids. The ECL values of 7 components of the 2 fatty acid methyl ester standards matched up with ECL values for peaks of stigmatic exudate and stylar flushes, and included: capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids. Stigmatic exudate contained 10 saturated fatty acids, 8 unsaturated fatty acids and 5 unknown peaks, while stylar fulshes produced 10

saturated fatty acids, 8 unsaturated and 3 unidentified peaks. Stigmatic exudate had 3 unknown peaks with ECL values of 12.60, 13.60 and 14.60 not found in stylar flushes. Stylar flushes produced 1 peak with an ECL value of 14.40 not obtained from stigmatic exudate. Equivalent chain length values 12.60, 13.60, 14.60, 15.40 and 18.98 were obtained for the unidentified peaks for stigmatic exudate. Stylar flushes revealed unknown peaks with ECL values of 14.40, 15.40 and 18.98. The eight carbon saturated fatty acid was generally covered up by the solvent peak and as a result no comparison between stylar flushes and stigmatic exudate were made. Peaks due to contamination from the lipid extraction procedure were not found.

The primary peaks obtained by gas chromatography of lipid extracts of stigmatic exudate and materials flushed from the stylar canals of heat-treated, DMSO-treated and aged styles were mostly long chain saturated and unsaturated fatty acids of 16 and 18 carbons in length, however, 9:0, 9:1, 12:0, 13:0, 14:0, unknown-2 and unknown-5 were also found in high concentration (Table 4). Oleic acid (18:1) predominated as the major peak for 'Nellie White' stigmatic exudate and usually for the 'Ace' and 'Nellie White' stylar flushes. The primary fatty acid for 'Ace' stigmatic exudate was highly variable resulting in high concentrations of 18:2, 16:0 and 14:0. Short chain fatty acids such as the nine carbon fatty acid were in high concentration in stylar flushes.

DISCUSSION

The lipid classes of stigmatic exudates for only a few species have been reported thus far, and so a definite chemotaxonomic pattern cannot be detected; however variability exists between flowering plants. Thin-layer chromatographic analysis of total lipid extracts of stigmatic exudate from Zea mays developed in hexane:diethyl-ether:acetic acid (90:10:1) revealed 11 neutral lipid spots (Table 3), including free fatty acids, triglycerides, esters and other unidentified spots (Martin 1969), as is the case with Lilium longiflorum stigmatic secretions. The major portion of the stigmatic exudate of Petunia hybrida is composed of pure fat, free from phospholipids, sterols and free fatty acids (Konar and Linskens 1966). Stigmatic exudate from Ipomoea batatas developed in hexane:diethyl-ether:acetic acid (90:10:1), or chloroform:methanol:water (75:22:3) resulted in 9 unidentified spots (Martin 1971).

The stigmatic secretions studied thus far indicates a wide range in the number of fatty acids present and in carbon chain length between species. In Ipomoea batatas (Martin 1971) 2 primary fatty acids were found (Table 4), caprate (10:0) and laurate (12:0), with traces of caprilate (8:0) and myristate (14:0); stigmatic exudate of Petunia hybrida contained 8 fatty acids with carbon chain lengths from 11 to 20 (Konar and Linskens 1966), 4 of which were components of Lilium longiflorum stigmatic exudate. Fourteen fatty acids were found in stigmatic exudate of Strelitzia reginae, ranging in carbon chain length from 8 to 18 (Martin 1970); 10 are common to L. longiflorum. Of the 12 fatty acids, ranging in carbon chain length from 6 to 18, obtained from stigmatic exudate of Zea mays (Martin 1969), 12 were found in the Easter lily.

The relative concentrations of the primary fatty acids were variable between experiments. Breakdown of fatty acids during freezer storage of

stigmatic exudate and stylar flushes prior to extraction could account for some of the differences in relative concentrations of the primary peaks. Also, thawing and refreezing of vials containing stigmatic exudate may explain a decrease in the unsaturated fatty acids as a result of oxidation.

In addition to the differences in the lipid patterns of stigmatic exudate and stylar flushes, sugar differences have also been reported by Loweus and Labarca (1973). The spots obtained on thin-layer plates each represent a class of lipids and within a particular class more than 1 lipid can occur. If specific lipid components of stylar flushes have properties suitable for the metabolism of a compatible pollen tube and thus is a much better food than the lipids of stigmatic secretions, then specific lipid components of stylar flushes at the class level will probably show differences in composition in comparison to stigmatic exudate. However, specific proteins and carbohydrates of stigmatic exudate and stylar flushes need to be identified in order to determine the role of lipids in the metabolism of the compatible pollen tube. If the biochemical patterns of proteins and carbohydrates are the same between stigmatic exudate and stylar flushes then we can conclude that lipids are important to compatible pollen tube growth. Differences in lipid patterns between stigmatic exudate and stylar flushes would indicate that these compounds may also play a role in compatible pollen tube growth and therefore are probably components of the speical stylar substance. The effectiveness of the special stylar substance in relation to compatible pollen tube growth may involve an additive effect of lipids, carbohydrates and proteins each providing a certain degree of the total nutrition required for the pollen tube to reach the ovary. By separately injecting lipid, protein

and carbohydrate extracts into the stylar canals of heat-treated flushed styles, followed by self or cross pollination, we could then determine the percent contribution of each group of compounds to compatible pollen tube growth.

The lipids flushed from the stylar canals of heat-treated, DMSO-treated and aged styles indicates that each releases identical lipid components as the other. Within a particular class, specific lipid components may also be the same among the stylar flushes. Since lipids flushed from heat-treated styles reveals the same biochemical patterns as materials flushed from DMSO-treated and aged styles, then we might conclude that all are special stylar substance. Injection of lipids flushed from DMSO-treated and aged styles into heat-treated flushed styles followed by self pollination would determine whether or not materials flushed from all stylar flushes are capable of supporting compatible pollen tube growth.

The chief fatty acids observed from stigmatic exudate and stylar flushes of Lilium longiflorum reveals a chemotaxonomic pattern similar to other genera studied in the same family. Within the family Liliaceae, palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) were found in seeds most frequently and in the greatest concentration in 10 genera analyzed (Gibbs 1974). Linolenic (18:3) and eicosenoic (20:1) were obtained infrequently and in low concentrations.

The lipids flushed from the stylar canal may be part of the special stylar substrate required for compatible pollen tube growth as proposed by Ascher (1966) in a gene action model explaining gametophytic self-incompatibility. This model states that after pollen tubes penetrate into the stylar canal, products of the stylar 'S' alleles move into the growing pollen tube where they encounter the products of the pollen 'S'

allele. When one of the two stylar 'S' allele products matches the product of the pollen 'S' allele, they unite forming a repressor which prevents pollen tubes from switching from a slow growth rate metabolism to a fast growth rate metabolism. The repressor may be blocking the production of enzymes essential for the fast growth rate metabolism, resulting in an inefficient utilization of the available stylar metabolites. The repressor could travel from the stylar canal secretory cells to the growing pollen tube via the stylar exudate and its molecular form may be that of a nucleic acid or a protein (Ascher 1974, Campbell and Ascher 1974, Ascher 1971). The fast growth rate metabolism is dependent on the presence of special stylar substrates (Ascher 1966). The slow growth rate metabolism utilizes pollen reserves and simple stylar components (Ascher 1966). Of the lipids flushed from the stylar canal, triglycerides and free fatty acids in particular are excellent sources of food which the pollen tube may need in order to reach the ovary.

Further analysis of individual spots obtained by thin-layer chromatography must occur in order to determine whether differences exist within spots, between stigmatic exudate and materials flushed from the stylar canal. Some spots may be simply hydrolysis products and not lipids (Personal communication:Klopfenstein). The effect of DMSO on materials flushed from the stylar canal needs to be researched in order to determine whether lipid patterns are altered from the use of this compound. Identification of specific lipid components of pistil exudate, followed by a bio-assay of these compounds in relation to their possible role in serving as a source of nutrition for the growing pollen tube must be explored. A bio-assay would involve the injection of specific lipid components into the style from stigmatic exudate and materials flushed from the stylar canal followed by either self or cross pollination.

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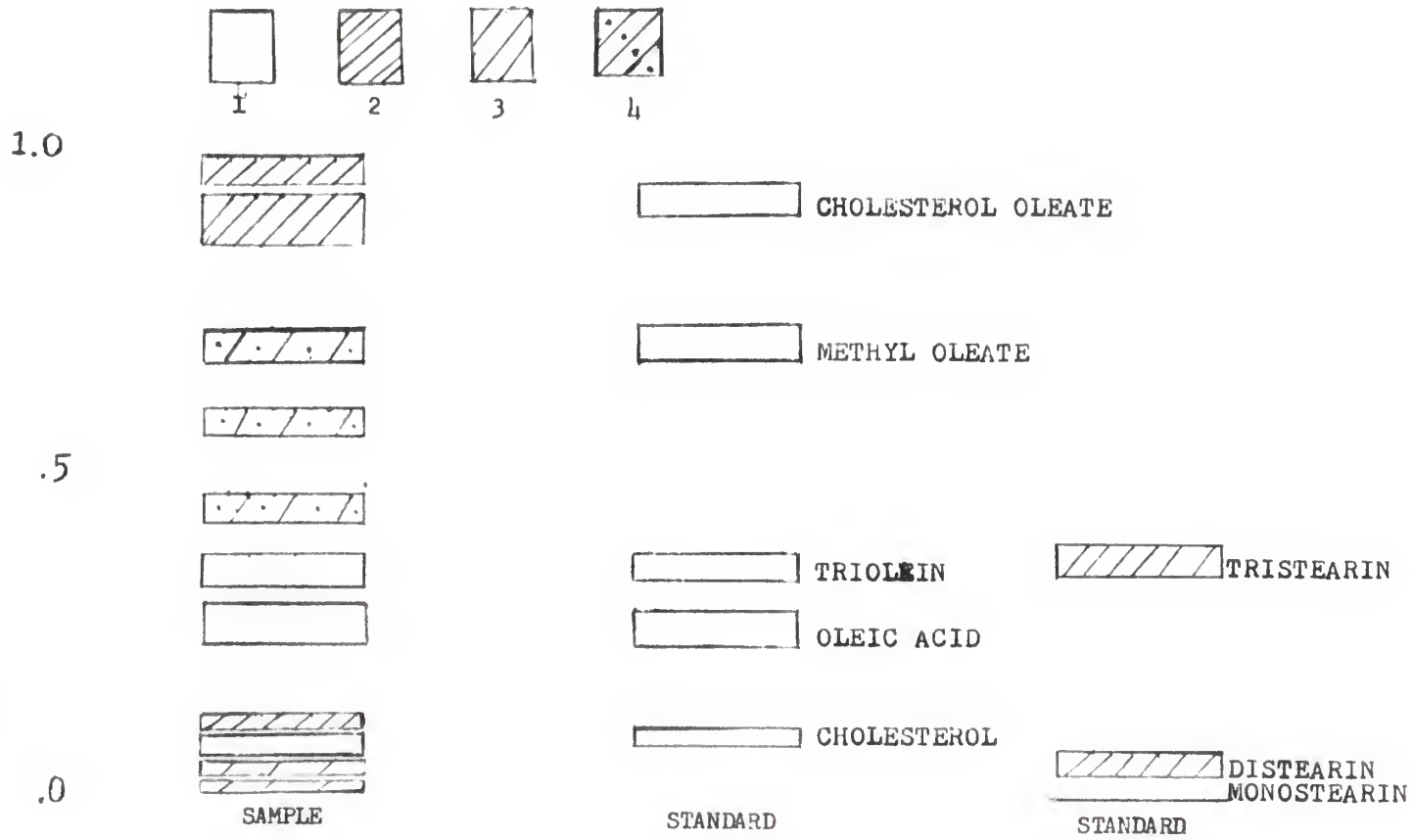
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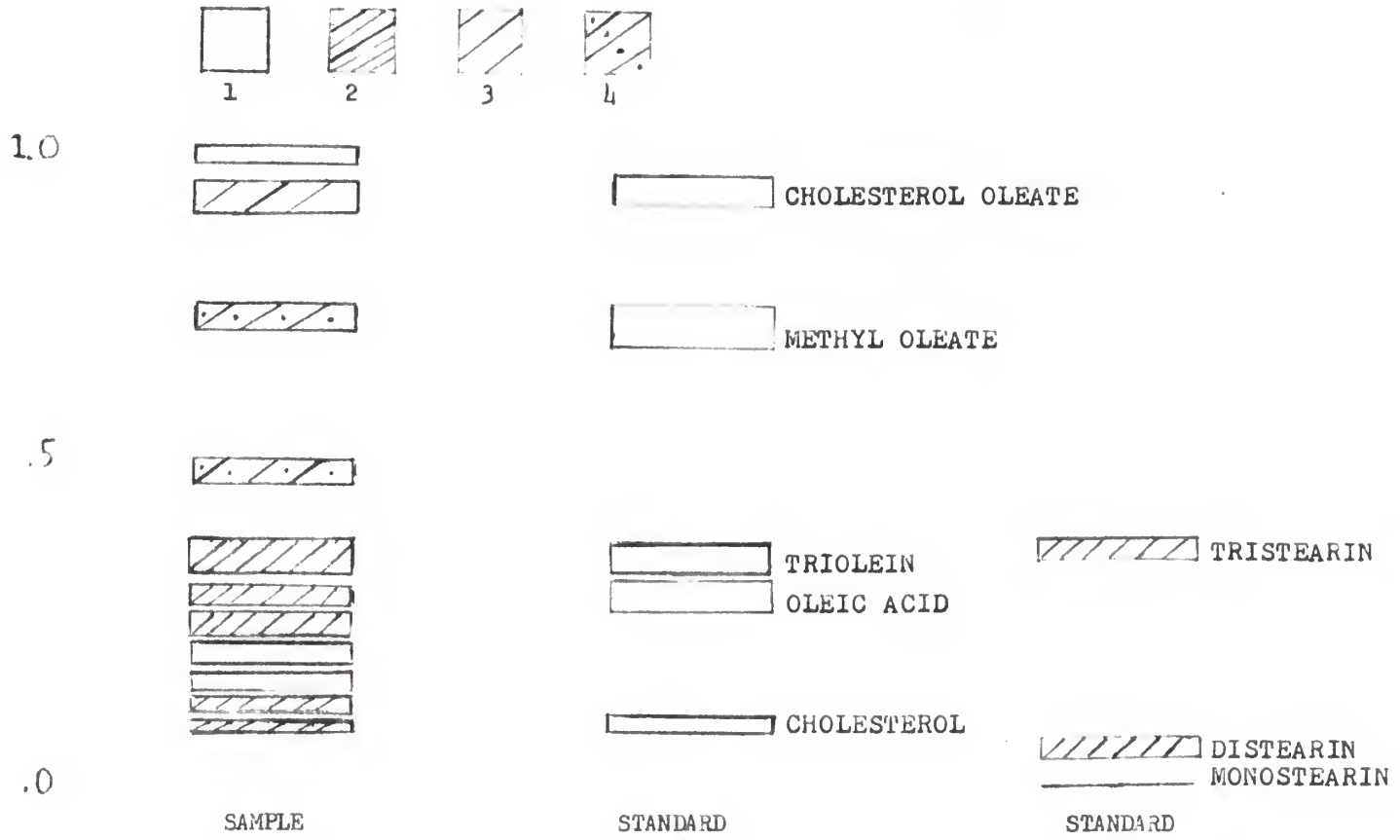
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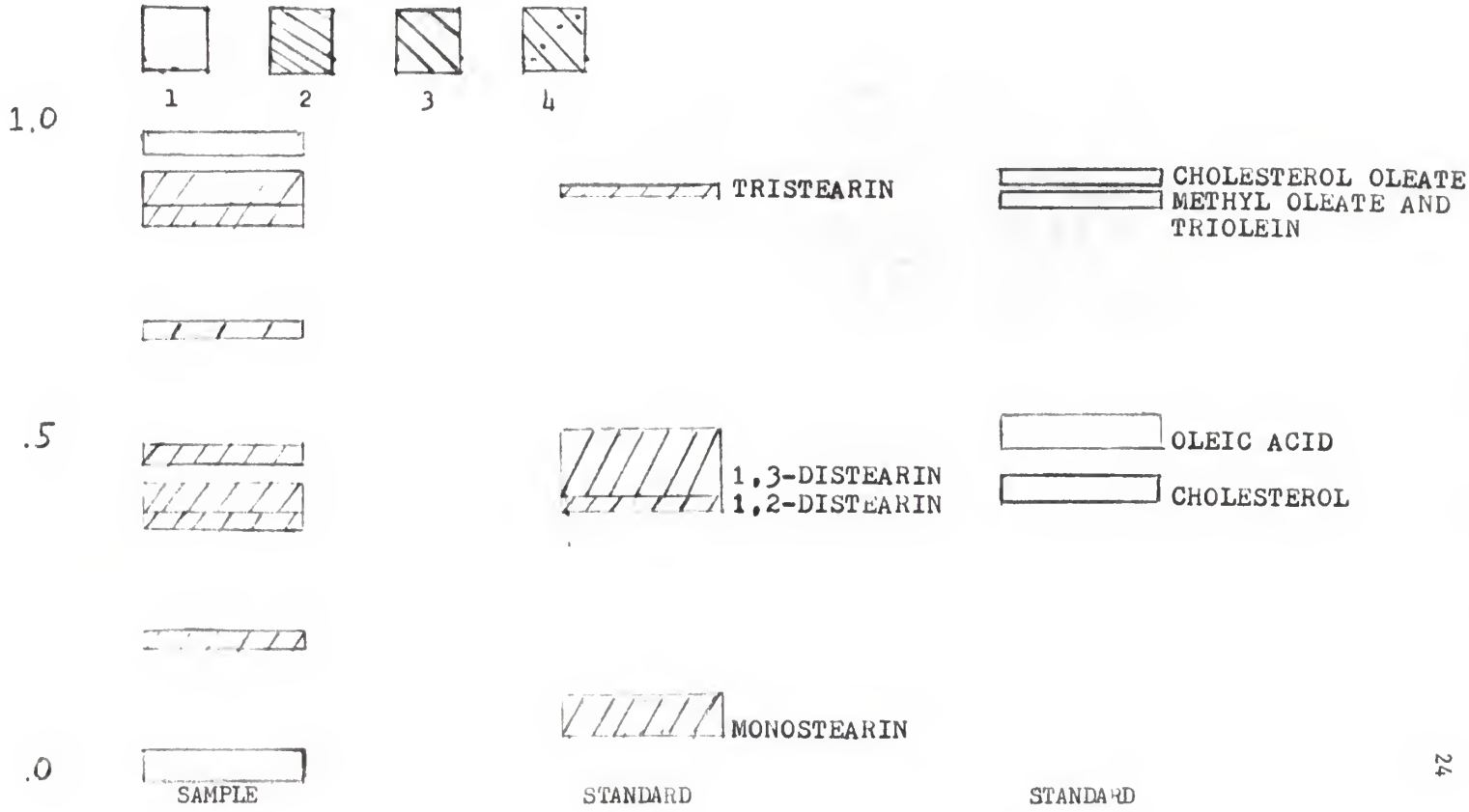
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1,2-DISTEARIN
OLEIC ACID
CHOLESTEROL
MONOSTEARIN
SAMPLE
STANDARD
STANDARD

Table 1

| | Self | Cross |
|---|---|---|
| Control | 45* | 90 |
| Water Injection | 45 | 90 |
| Heat Treated (50 C for 5 min) | 90 | 90 |
| Heat-Treated-Flushed | 45 | 45 |
| Heat Treated-Flushed-Injected | 90 | 90 |
| Prepollination Injection of Stigmatic Exudate into Heat Treated Flushed Styles | 45(?) | 45 |
| Stigmatic Exudate, Non Heat Treated | 50 | 95 |
| Dimethyl Sulfoxide Treated (10% DMSO for 10 minutes) | 40 | 90 |
| Aged | 90 | 90 |

*Sources of data: Ascher 1975; Hopper, Ascher, Peloquin 1967; Ascher and Drewlow 1971; Ascher and Peloquin 1966; Ascher 1971.

Table 2

| | GAS-LIQUID CHROMATOGRAPHY | | THIN LAYER CHROMATOGRAPHY | |
|---|---|---|---|---|
| | Stigmatic Exudate | Stylar Flushes | Stigmatic Exudate | Stylar Flushes |
| Amount of Exudate Used: | 1 ml | 20-25 ml | 2.5 ml | 40-45 ml |
| Amount of Extraction Solvent Used: | 60 ml | 80 ml | 60 ml | 90 ml |
| Virtis Flask Size: | 165 ml (#16-207) | 165 ml (#16-207) | 165 ml (#16-207) | 285 ml (#16-054) |
| Homogenization Time: | 3 min | 5 min | 3 min | 5 min |
| Size of Separatory Funnel: | 125 ml | 125 ml | 125 ml | 500 ml |
| Amount of $H_2O$ Used for Separation of $CHCl_3$ from $CH_3OH$: | 30 ml | 10 ml | 30 ml | 10 ml |
| Time Required for Separation of Extraction Solvent into 2 Layers: | 5 min | 5 min | 5 min | 15 min |
| Amount of $CHCl_3$ Drawn Off from Separatory Funnel: | 35 ml | 40 ml | 35 ml | 50 ml |

Table 3

| STANDARD FATTY ACID | STANDARD EQUIVALENT CHAIN LENGTH (ECL) | EXPERIMENTAL ECL VALUES LOG OF RETENTION TIME VERSUS CARBON NUMBER | CALCULATED ECL VALUES | FATTY ACID METHYL ESTERS STIGMATIC EXUDATE | FATTY ACID METHYL ESTERS STYLAR FLUSHES |
|---|---|---|---|---|---|
| 8:0 | 8.0 | 8.0 | 8.0 | | 8:0 |
| | | 9.0 | 9.0 | 9:0 | 9:0 |
| | | 9.61 | 9.62 | 8:1 | 8:1 |
| 10:0 | 10.0 | 10.0 | 10.0 | 10:0 | 10:0 |
| | | 10.42 | 10.42 | 9:1 | 9:1 |
| | | 11.0 | 11.0 | 11:0 | 11:0 |
| | | 11.43 | 11.42 | 10:1 | 10:1 |
| 12:0 | 12.0 | 12.0 | 13.0 | 12:0 | 12:0 |
| | | 12.60 | - | -* | |
| | | 13.0 | 13.0 | 13:0 | 13:0 |
| | | 13.60 | - | - | |
| 14:0 | 14.0 | 14.0 | 14.0 | 14:0 | 14:0 |
| | | 14.40 | - | | - |
| | | 14.60 | - | - | |
| | | 15.0 | 15.0 | 15:0 | 15:0 |
| | | 15.40 | - | - | - |
| 16:0 | 16.0 | 16.0 | 16.0 | 16:0 | 16:0 |
| | | 16.55 | 16.55 | 16:1 | 16:1 |
| | | 17.0 | 17.0 | 17:0 | 17:0 |
| | | 17.50 | 17.50 | 16:2 | 16:2 |
| 18:0 | 18.0 | 18.0 | 18.0 | 18:0 | 18:0 |
| 18:1 | 18.47 | 18.48 | 18.47 | 18:1 | 18:1 |
| | | 18.98 | - | - | - |
| 18:2 | 19.30 | 19.30 | 19.30 | 18:2 | 18:2 |
| | | 19.43 | 19.43 | 18:2(isomer) | 18:2(isomer) |
| 18:3 | 20.40 | | 20.40 | | |

*Unknown

Table 4

| Cultivar: | EXPERIMENT | | STIGMATIC | HEAT | DMSO | AGED |
|---|---|---|---|---|---|---|
| ACE | 1 | 1. | 18:2 | 18:1 | 18:1 | 18:1 |
| | | 2. | 16:2 | 16:1 | 18:0 | 16:0 |
| | | 3. | 18:1 | 16:0 | 16:0 | 9:0 |
| | | 4. | 16:1 | 18:0 | 16:1 | 14:0 |
| | 2. | 1. | 14:0 | 18:1 | 18:1 | 18:1 |
| | | 2. | 18:1 | 18:0 | 9:1 | 16:0 |
| | | 3. | 16:0 | 16:2 | 13:0 | 16:1 |
| | | 4. | 18:0 | 16:0 | 18:0 | 16:2 |
| | 3. | 1. | 16:0 | 16:0 | 18:1 | 18:1 |
| | | 2. | 18:1 | 18:1 | 16:0 | 16:0 |
| | | 3. | 14:0 | 14:0 | 16:1 | 18:0 |
| | | 4. | 18:0 | 16:1 | 18:0 | 16:1 |
| NELLIE WHITE | 1. | 1. | 18:1 | 18:1 | 18:1 | 18:1 |
| | | 2. | 16:0 | UNK | 16:0 | 16:1 |
| | | 3. | 18:0 | 16:0 | 18:0 | 16:0 |
| | | 4. | 16:2 | 14:0 | 15:0 | 18:0 |
| | 2. | 1. | 18:1 | 18:1 | 18:1 | 18:1 |
| | | 2. | 16:2 | 18:0 | 18:2 | 16:0 |
| | | 3. | 18:2 | 16:1 | 18:0 | 18:2 |
| | | 4. | 16:0 | 16:0 | 16:0 | 16:1 |
| | 3. | 1. | 18:1 | 16:0 | 18:1 | 16:0 |
| | | 2. | 18:0 | 18:1 | 18:0 | 18:2 |
| | | 3. | Unknown | 14:0 | 16:1 | 18:1 |
| | | 4. | 16:1 | 16:1 | 16:0 | 16:1 |
| | 4. | 1. | 18:1 | | | 18:1 |
| | | 2. | 16:0 | | | 18:2 |
| | | 3. | 18:0 | | | 16:0 |
| | | 4. | 16:2 | | | 9:1 |
| | 5. | 1. | | | | 16:0 |
| | | 2. | | | | 12:0 |
| | | 3. | | | | 14:0 |
| | | 4. | | | | 9:1 |

Table 5

| Lipid Class | *Lilium longiflorum* | | *Petunia hybrida* | *Zea mays* |
|---|---|---|---|---|
| | Stigmatic | Stylar | | |
| Phospholipid | - | - | - | - |
| Glycolipid | - | - | - | - |
| Sterols | + | + | - | - |
| Free Fatty Acids | + | + | - | + |
| Diglycerides | - | + | +(?) | - |
| Monoglycerides | - | - | +(?) | - |
| Triglycerides | + | + | +(?) | + |
| Methyl Esters | + | + | - | - |
| Sterol Esters | + | + | - | - |
| Hydrocarbon Waxes | ? | ? | - | + |

Table 6

| Fatty Acids | Strelitzia reginae | Zea mays | Petunia hybrida | Ipomoea batatas | Lilium longiflorum |
|---|---|---|---|---|---|
| 6:0 | | 6:0 | | | |
| 8:0 | 8:0 | 8:0 | | 8:0 | |
| 9:0 | | | | | 9:0 |
| 8:1 | | | | | 8:1 |
| 10:0 | 10:0 | 10:0 | | 10:0 | 10:0 |
| 9:1 | | | | | 9:1 |
| 11:0 | | | 11:0 | | 11:0 |
| 10:1 | | | | | 10:1 |
| 12:0 | 12:0 | 12:0 | | 12:0 | 12:0 |
| Unknown (UNK) | | | | | UNK |
| 13:0 | | 13:0 | | | 13:0 |
| Unknown | | | | | UNK |
| 14:0 | 14:0 | | | 14:0 | 14:0 |
| 14:0(isomer) | | | | | |
| Unknown | | | | | UNK |
| 15:0 | 15:0 | | 15:0 | | 15:0 |
| Unknown | | | | | UNK |
| 16:0 | 16:0 | 16:0 | | | 16:0 |
| 16:1 | 16:1 | 16:1 | | | 16:1 |
| 16:2 | | | | | 16:2 |
| 16:3 | | | 16:3 | | |
| 17:0 | 17:0 | 17:0 | 17:0 | | 17:0 |
| 18:0 | 18:0 | 18:0 | | | 18:0 |
| 18:0(isomer) | 18:0 | | | | |
| 18:1 | 18:1 | 18:1 | | | 18:1 |
| Unknown | | | | | UNK |
| 18:2 | 18:2 | 18:2 | 18:2 | | 18:2 |
| 18:2(isomer) | | | | | 18:2 |
| 19:0 | | | 19:0 | | |
| 18:3 | 18:3 | 18:3 | 18:3 | | |
| 20:0 | | | 20:0 | | |

# LIPIDS OF PISTIL EXUDATES AND SELF INCOMPATIBILITY IN *LILIUM LONGIFLORUM* THUNB.

by

ROBERT WILLIAM HADEL JR.

B.S. University of Missouri at Kansas City, 1974

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1977

Thin-layer chromatography of lipid classes of stigmatic exudate using a solvent system composed of chloroform:methanol:water (65:25:4) resulted in 2 spots with $R_f$ values of 0.93 and 1.0, while the 3 stylar flushes each had spots with $R_f$ values of 0.75, 0.93 and 1.0. Thin-layer chromatography of lipid classes from stigmatic exudate and the 3 stylar flushes using hexane:diethyl-ether:acetic acid (90:10:1) resulted in 11 spots for each extract; however, some spots were different. Gas chromatographic analysis of fatty acids from L. longiflorum stigmatic exudate revealed 23 peaks, while materials flushed from the hollow style of heat-treated, DMSO-treated and aged styles produced 21 peaks.